

Observations on the pathogenesis of nasal priming

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To understand better the response of patients with allergic rhinitis to nasal challenge with antigen, we studied the mechanism of priming, that is, the increased clinical response to daily sequential nasal challenges. Ten subjects with ragweed hay fever were challenged four times with increasing doses of ragweed pollen. The first 2 challenge days were separated by 2 weeks, whereas the last three challenges occurred on sequential days. Nasal lavages, performed before and after each nasal challenge, were evaluated for levels of inflammatory mediators and cellular content. In contrast to control days, a significant ($p < 0.05$) increase in the number of sneezes occurred on both priming days. Priming was associated with a significant increase in the level of histamine on both priming days, whereas the second priming day was also associated with an increase in TAME-esterase activity, kinins, and prostaglandin D_2 obtained after challenge ($p < 0.05$ for all). In the lavages before challenge on the priming days, the total number of cells and the number of neutrophils, eosinophils, and alcian blue-positive cells were significantly increased, but in contrast, basal levels of mediators were not. The net increase in the number of alcian blue-positive cells correlated with the net increase in the amount of histamine released on the priming days ($r = 0.661$; $p < 0.05$). These studies suggest that priming results, in part, from increased mediator release from influxing inflammatory cells. (J ALLERGY CLIN IMMUNOL 1989;84:492-501.)

That the response of an allergic individual to pollen inhalation is more than the immediate anaphylactic release of mediators from mast cells was first noted when investigators, performing nasal challenges with antigen, observed that the number of pollen grains necessary to induce an immediate allergic reaction in the nose of an asymptomatic individual exceeded the number of pollen grains inhaled within an equivalent

Abbreviations used

LTC₄: Leukotriene C₄
PGD₂: Prostaglandin D₂

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period of time during seasonal exposure. To reconcile the discrepancy, Connell¹ challenged subjects daily with ragweed pollen and found that they required lower doses of pollen on successive days to increase their nasal airway resistance. After several days of exposure, the amount of pollen needed to induce a clinically apparent reaction decreased, approaching levels commonly encountered during seasonal exposure, a phenomenon he defined as priming. Connell² extended this initial observation by demonstrating in a limited number of patients, that priming (1) was specific for the side of the nose challenged, (2) disappeared when exposure was stopped, (3) occurred during seasonal exposure, and (4) was not specific for the antigen inhaled; that is a subject could be primed by exposure to one antigen for a second antigen to which he was known to be allergic. Bacon et al.³ reproduced this observation with ragweed extract instead of pollen and suggested that priming was specific for antigen, since subjects did not prime to an irritant,

ammonia. Borum et al.,⁴ as well as Konno et al.,⁵ have also observed this phenomenon. Although priming has had great impact on the design of nasal challenge experiments, observations on understanding its pathogenesis are surprisingly limited.

Besides the necessity of challenging with large doses of pollen antigens for induction of an immediate allergic reaction in asymptomatic individuals, other observations suggest that the immediate anaphylactic response inadequately explains the pathogenesis of allergic rhinitis. Pelikan⁶ described the spontaneous recurrence of airway obstruction hours after a nasal challenge. Events occurring within this time frame are usually referred to as late reactions. Although late reactions have been more extensively studied in the skin and lungs of humans, as well as animals, late reactions after nasal challenge with antigen are also documented.⁷⁻¹⁶ During studies of the nasal late reaction, we began to challenge patients with antigen 10 hours after an initial reaction. Besides observing a pattern of mediator release similar to the early reaction, we observed that the response was frequently augmented in terms of mediators and symptoms.¹⁷ We also observed that the oral administration of prednisone, a drug effective in treating the seasonal symptoms of allergic rhinitis, inhibited the late reaction as well as the augmented response.¹⁸ To determine if similarities exist between the rechallenge reaction and priming and to explore the potential pathogenetic mechanisms of priming, we evaluated the cellular and mediator content of nasal lavages of individuals before and after they were challenged on consecutive days with ragweed pollen.

METHODS

Study design

Volunteers with a history of ragweed hay fever and a positive intradermal skin test to ragweed-antigen extract at a concentration of 1 PNU/ml or less came to the laboratory on four separate occasions. The first and second visits were separated by 2 weeks, whereas the second, third, and fourth visits occurred on sequential days. The first and second visits served to control for the reproducibility of the challenge procedure, whereas the sequential challenges evaluated priming. The 2-week time period separating control challenges was based on prior experience. Each patient was asymptomatic when the series of nasal challenges was started and was not predicted to become symptomatic from environmental exposure during the study, based on history and the response to a battery of intradermal skin tests.

Nasal challenges

During each visit, the patients underwent a nasal challenge with ragweed pollen. The protocol began with nasal lavages performed four times with 10 ml of lactated Ringer's solution to determine the level of mediators and the cellular

content of nasal secretions before pollen exposure. Next, 0.1 ml of a 0.05% solution of oxymetazoline was applied to each nostril to prevent the mucosal edema that follows a positive response to antigen challenge and that interferes with subsequent lavages. The volunteers were then challenged twice with 25 mg of lactose powder and then four times with increasing amounts of ragweed pollen (10, 100, 1000, and 10,000 grains). Each challenge with pollen or lactose was separated by 12 minutes. A nasal lavage was performed 10 minutes after each challenge, with an additional lavage performed 20 minutes after the 10,000 grain pollen challenge to demonstrate that the level of mediators was declining.

The pollen grains and lactose were prepared as previously described.¹⁹ Gelatin capsules were preloaded with 25 mg of lactose or a mixture of pollen and lactose made to contain the desired number of pollen grains in 25 mg. The 10,000 grain pollen capsule contained 0.025 mg of pollen; 1000 grain, 0.0025 mg; 100 grain, 0.00025 mg; and 10 grain capsule, 0.000025 mg of pollen. The contents of the capsules were delivered to the patient by placing them in a Spinhaler (Fisons Corp., Rochester, N.Y.) connected to an air tank that had an intervening dosimeter that regulated the amount of air directed through the Spinhaler. The other end of the inhaler was adapted to fit into a nostril. The challenges with lactose tested for a nonspecific reaction induced by the delivery system. Additionally, three subjects were challenged on 3 consecutive days, with lactose being substituted for all the ragweed-pollen capsules to assure further that the results obtained were not merely the effect of prolonged irritation induced by the delivery system.

Lavages

The subjects had nasal lavages performed in a sitting position with the head extended 45 degrees from the horizontal. After 10 seconds, the patient expelled the lavage into a plastic receptacle. The recovered fluid from the lavage was transferred to a polypropylene tube and stored on ice until the completion of the experiment. The first lavage was centrifuged at 15,000 g for 15 minutes. The supernatant was processed for mediators as described below. The cell pellet was resuspended in Hanks' buffer. The next three nasal lavages were processed similarly, with the exception that the supernatants were discarded. The cells from the first four lavages were combined. The pellets were incubated with 10% *N*-acetyl-L-cysteine for 45 minutes at 37° C to dissolve the mucus, permitting the attainment of single cell suspensions. The cells were washed three times in Hanks' balanced salt solution, divided into aliquots, and placed in a cytocentrifuge. A minimum of one slide was stained with Diff-Quick (American Scientific Products, McGraw Park, Ill.) (a modified Wright stain) and one slide with alcian blue, pH 1. The cell-staining techniques have been used before and are described in greater detail elsewhere.²⁰ The lavage after the oxymetazoline was discarded. We discarded the supernatants from the second through the fifth lavages because thousands of prior experiments have demonstrated that the mediator levels only decrease or remain stable. The reduction in the number of the samples assayed permitted

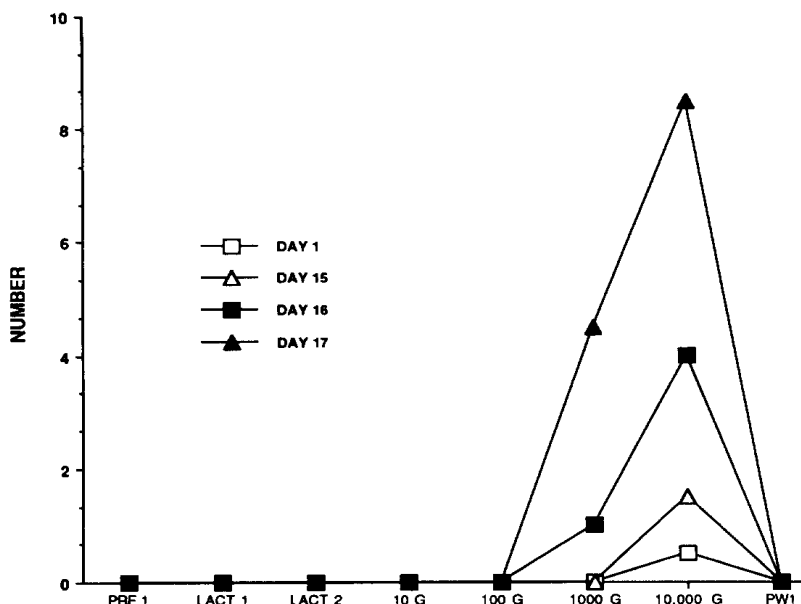


FIG. 1. The median number of sneezes occurring within 10 minutes after each part of the nasal challenge. The time between beginning and completion of the nasal challenge is approximately 100 minutes. *Open symbols* represent challenges performed on the control days, whereas *closed symbols* represent those occurring on the priming days. *Pre*, before challenge; *lact*, lactose; *post*, 20 minutes after last challenge. For an explanation of statistical comparison, see the Methods section.

all the samples from a given subject to be measured in the same assay, thus limiting the effect of interassay variability. The lavages occurring after the lactose and pollen challenges were handled as described before.^{19, 21, 22} In brief, the lavages were centrifuged at 15,000 *g* for 15 minutes to remove the cells. The supernatants were processed for the measurement of histamine, TAME-esterase activity, kinins, LTC₄, and PGD₂. Eight tenths of a milliliter of supernatant, to be used for the measurement of histamine, was combined with 0.2 ml of 8% perchloric acid and was then frozen at -20°C . Before assaying, the samples were thawed and centrifuged, and the supernatant was assayed for histamine. The samples for TAME-esterase activity were also frozen to -20°C . The samples (0.5 ml) for PGD₂ and LTC₄ were mixed with 95% ethanol in a ratio of one part sample to four parts ethanol. The samples for kinin determination were made 40 mmol/L with respect to ethylenediaminetetraacetic acid. All samples for the determination of kinins, PGD₂, and LTC₄ were stored at -80°C until they were assayed. The mediators measured are all stable for months under the storage conditions described. Because of limitations in the amount of sample returned, insufficient material existed to perform additional analytical analyses on the leukotrienes, prostaglandins, TAME-esterase activity, and kinins recovered. In previous experiments, the TAME-esterase activity in the early reaction to nasal challenge was demonstrated to represent approximately 75% plasma kallikrein, 20% mast cell tryptase, and <5% glandular kallikrein.^{23, 24} The leukotrienes detected in the early reaction of asymptomatic subjects challenged out of season are predominately LTC₄ with

some LTD₄,¹⁹ whereas the kinins are a mixture of lysylbradykinin and bradykinin.²² Samples that contained levels of mediators less than the sensitivity of the assay were reported as the minimal detectable dose of the assay used, that is, 1 ng/ml for histamine,²¹ 39 pg/ml for kinins,²² 1000 cpm for TAME-esterase activity,²¹ 250 pg/ml for LTC₄,¹⁹ and 20 pg/ml for PGD₂.²¹

Statistical methods

Nonparametric Wilcoxon paired rank-sums tests were used to compare the response on day 15 to the other days. We compared the net increase above baseline for the antigen challenge; that is, the mean response after the lactose challenges was subtracted from the response to each dose of antigen before summing the levels from all points on the dose-response curve. Two-tailed tests were used, and $p < 0.05$ was considered significant. Associations between variables was evaluated by Spearman rank correlations.

RESULTS

To study priming we investigated whether an allergic subject's sensitivity to antigen increases after a recent exposure to the same antigen. The group data for sneezing indicate that priming occurred as follows (Fig. 1): On the control days (1 and 15), the responses were similar, and most subjects did not begin to sneeze until they inhaled 10,000 grains of ragweed pollen. In contrast, on the priming days (16 and 17) the total number of sneezes occurring during the challenge in-

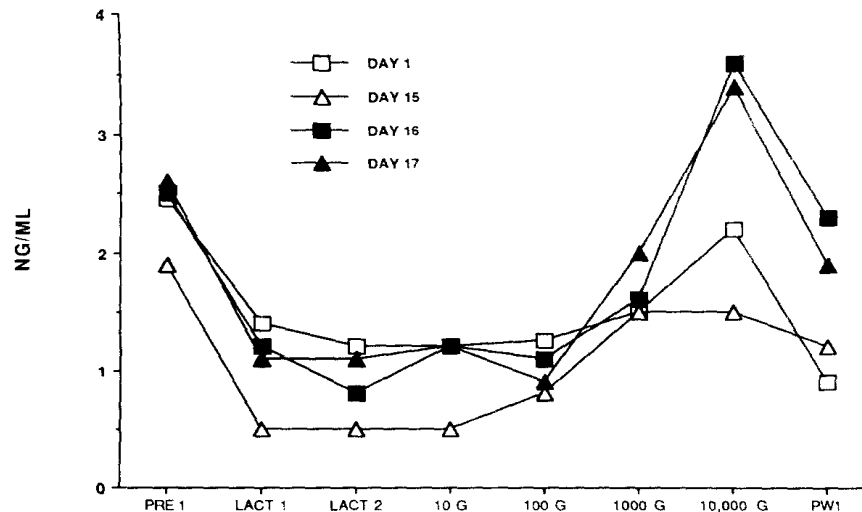


FIG. 2. Results for level of histamine in recovered nasal lavages. Symbols same as in Fig. 1.

creased significantly ($p < 0.05$). In addition, the threshold for a reaction was shifted toward a smaller dose of pollen, and the magnitude of the response was increased at equivalent doses. Several subjects demonstrated a response to 100 grains of pollen on days 16 or 17. Six of the 10 patients demonstrated the pattern of sneezing response described for the group. The other four subjects had little change in response during the 4 challenge days, but no subject demonstrated decreased responsiveness. Scores obtained by diary demonstrated a similar pattern but did not achieve statistical significance, presumably because premedication with oxymetazoline interferes with the subjective assessment of congestion and nasal lavage interferes with the sensation of rhinorrhea. The three individuals challenged with six doses of lactose on 3 consecutive days did not sneeze, report changes in symptom score, or produce mediators.

We measured histamine, TAME-esterase activity, kinins, PGD_2 , and LTC_4 before and after challenge with antigen. The only mediator to demonstrate a significant difference in the level present in the first lavage before challenge was kinin. The level on day 15 (68 pg/ml, median) was significant ($p < 0.05$), although it was slightly less than levels on day 1 (101 pg/ml), day 16 (224 pg/ml), and day 17 (257 pg/ml). As previously described, baseline levels of mediators were lowered by several prechallenge lavages.²¹ After antigen challenge, the amount of mediators increased over lactose challenge and increased more on days 16 and 17, indicating that priming occurred. Histamine levels increased only after the 10,000 grain challenge on days 1 and 15 (Fig. 2). The amount of histamine released at the 10,000 grain

dose was greater on the priming days, 16 and 17. The total increase in the level of histamine on days 16 and 17 was significantly greater than on day 15 (Table I).

The pattern of the response for TAME-esterase activity (Fig. 3), kinins (Fig. 4), PGD_2 (Fig. 5), and LTC_4 (Fig. 6) demonstrated a similar pattern to histamine and sneezing, but the degree of significance was different. The amount of these mediators generated on the control days 1 and 15 were similar. The response on day 16, the first priming day, was greater for each but did not achieve statistical significance. On day 17, however, there was a significant increase in the total amount of TAME-esterase activity, kinins, and PGD_2 (Table I). Although the response for immunoreactive LTC_4 also was augmented, the difference on day 17 was not significant.

There were few cells in the nasal lavages obtained before the challenges on control days (Fig. 7). Twenty-four hours after the challenge on day 15, there was a tenfold increase in the total number of white blood cells recovered, with the number of neutrophils, eosinophils, and alcian blue-positive cells all being increased. Epithelial cells did not increase (data not presented). Total cell numbers did not increase further in the day 17 prechallenge specimen. On the average, neutrophils represented about 60% of the total white cells; eosinophils, 25%; alcian blue-positive cells, 1%; with the remainder being mononuclear cells not further characterized.

As the number of cells increased on the priming days, we examined the relationship between the cellular influx and priming by correlating the net increase in the number of cells with the net increase in the number of sneezes, symptom scores, and mediator

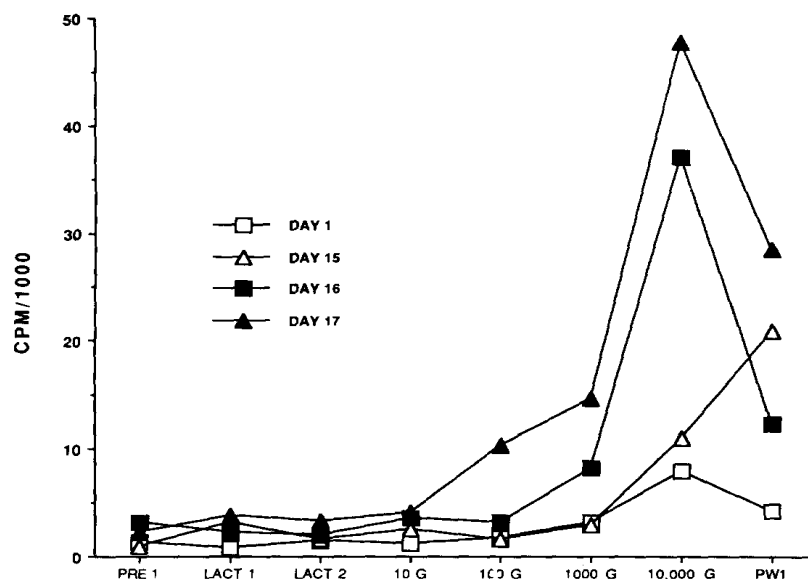


FIG. 3. Results for level of TAME-esterase activity in recovered nasal lavages. Symbols same as in Fig. 1.

TABLE I. Response to antigen challenge*

	Day 1			Day 15			Day 16			Day 17		
	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min
Sneezes (No.)	2	12	0	4	19	0	10.5	30	0	10	41	4
Histamine (ng/ml)	-0.48	7.5	-9.1	1.2	5.2	-18.5	3.8	15.1	-2.1	3.4	28.3	-6.1
TAME (cpm $\times 10^{-3}$)	9.2	233	-13	11.7	276	-1.2	59	371	0.8	99	354	0.65
Kinin (ng/ml)	0.42	6.08	0	0.77	7.73	-0.06	2.35	8.21	0	5.08	10.5	0.02
PGD ₂ (pg/0.1 ml)	7.5	73	0	9.3	54	-29	16.8	196	-30	50	386	0
LTC ₄ (ng/ml)	7.7	115.2	0	12.7	85.9	0	32.4	165.9	-19	32.6	236	0.65

Med, Median; Max, maximum; Min, minimum; TAME, TAME-esterase activity.

*Response is represented as net change from baseline (for details, see Methods section).

levels. Increase in symptom scores, but not number of sneezes, correlated significantly ($p < 0.05$) with the number of alcian blue-positive cells ($r = 0.648$) and mononuclear cells ($r = 0.685$), and nearly significantly with neutrophils ($r = 0.524$) and eosinophils ($r = 0.524$). Net increase in the number of alcian blue-positive cells on the priming days correlated with net increases in histamine levels on the priming days ($r = 0.661$; $p < 0.05$). Despite significant correlations ($p < 0.05$) between changes in histamine, TAME-esterase activity, and kinins, there were no significant correlations between changes in the levels of mediators and either changes in the number of cells or symptoms. The changes in leukotrienes and PGD₂

did not correlate with any of the parameters assessed. The limited number of significant correlations found may be due to the small sample size.

DISCUSSION

Much information about the allergic response has been acquired by challenging asymptomatic allergic subjects intranasally with antigen. Is challenging an asymptomatic allergic subject out of season equivalent, however, to the multiple repetitive challenges that occur during seasonal exposure? Exposure to antigen induces an inflammatory response that persists beyond the 30-minute symptomatic, immediate, anaphylactic response. An example is the occurrence of late-phase

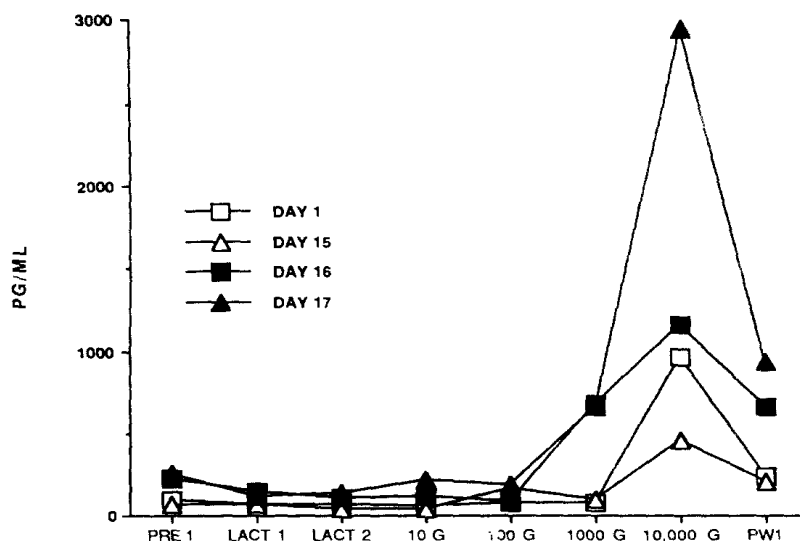


FIG. 4. Results for level of kinins in recovered nasal lavages. Symbols same as in Fig. 1.

<i>p</i> Values		
Day 15 vs 1	Day 15 vs 16	Day 15 vs 17
0.054	0.02	0.01
0.38	0.03	0.04
0.17	0.1	0.01
0.4	0.48	0.01
0.5	0.24	0.01
0.29	0.48	0.17

symptoms during the 12 hours after antigen challenge in approximately 50% of subjects with ragweed hay fever²⁵ that is accompanied by some inflammatory mediators and a cellular influx.^{20, 26}

An increase in reactivity to histamine and methacholine has also been demonstrated to follow antigen exposure.^{27, 28} Priming, that is, an increase in the response to the same quantity of antigen, is another manifestation of the change in the reactivity of the nasal mucosa that can follow antigen exposure. The present investigation was directed toward understanding the pathogenetic mechanism of priming.

We revealed priming in our subjects by demonstrating that reactivity to antigen, as manifested by sneezing on 2 days separated by 2 weeks, was equivalent,

whereas reactivity 24 hours after antigen exposure was significantly increased. This increase in sensitivity to antigen occurred in most subjects (6/10). Whether priming would have occurred in every subject with more prolonged antigen exposure was not evaluated. The use of smaller increments in the dose of antigen (i.e., less than tenfold increases) may have demonstrated a change in threshold in a greater number of subjects. Not every subject may have a priming response. Subjects who do may become more ill during seasonal exposure.

On the control days, most subjects either did not react or reacted only to the challenge with 10,000 grains of ragweed pollen. In Baltimore at the peak of the ragweed hay fever season, it is estimated that a subject breathing at tidal volume will inhale 150 grains of pollen per hour.²⁹ The discrepancy between the amount of pollen inhaled during the season and the amount needed to induce a response in an asymptomatic individual in the laboratory means that priming must be invoked to explain seasonal symptoms. Several of our subjects, once they had been exposed, now responded to 100 grains of pollen, an amount easily inhaled within minutes during seasonal exposure. Although we report that 100 grains of pollen was placed in the capsule, we estimate that about 60% actually lands on the nasal mucosa. Some grains of pollen invariably remain in the capsule after actuation, whereas other grains may not be captured by the mucosal because of the delivery system. Thus, the dose of pollen necessary to induce a symptomatic re-

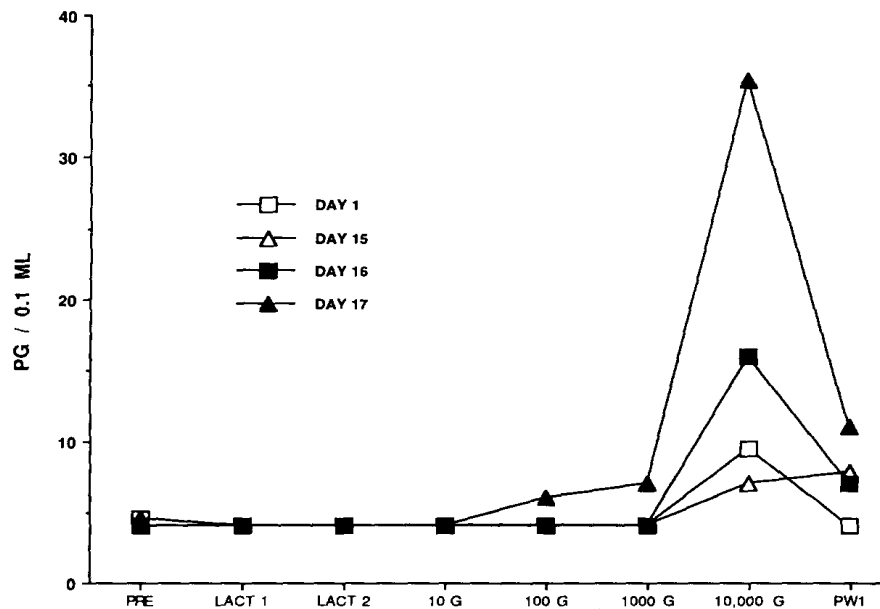


FIG. 5. Results for level of PGD₂ in recovered nasal lavages. Symbols same as in Fig. 1.

sponse after priming closely parallels seasonal exposure.

We erroneously reported that doses of 10 grains of pollen induced a response in asymptomatic patients.^{19, 21, 22, 30} This mistake was the result of an error in calculating the conversion of milligrams of pollen to number of grains of pollen. The previously reported dose of 10 grains of pollen was actually equivalent to 10,000 grains of pollen used in the experiments herein described. Our present study demonstrated that none of the 10 asymptomatic allergic subjects released inflammatory mediators in response to <10,000 grains of pollen. As reported here, however, subjects can release inflammatory mediators to 100 grains of pollen if they are exposed to antigen 24 hours before rechallenge.

One explanation for the increased amount of mediators after priming is the presence of additional target cells. Alcian blue-positive cells present in the nasal lavages before the challenges on the priming days would be expected to release histamine and other mediators on exposure to antigen. These cells are probably basophils, as described during the late-phase reaction and during seasonal exposure.^{20, 31-33} Support for this speculation comes from the fact that the level of histamine increased the first priming day, whereas the amount of PGD₂ did not. Human lung mast cells release PGD₂ and histamine, whereas peripheral blood basophils release histamine but no appreciable amount of PGD₂.²⁶ Thus, the increased amount of histamine

on the first priming day could reflect the response of the same number of mast cells plus additional histamine from basophils. Further support for this hypothesis is the correlation between the net increase in the number of alcian blue-positive cells and the net increase in histamine on the control and priming days. Similar results were observed in the rechallenge response described at 12 hours.¹⁸

The increase in PGD₂ on the second priming day may indicate the migration of a subpopulation of mast cells. In the skin, Mitchell et al.³⁴ demonstrated that basophils migrated to denuded areas of skin during the first 2 days, whereas mast cells migrated later. Also, Enerbach et al.³⁵ demonstrated that seasonal exposure led to the migration of mast cells toward the epithelium or the nasal mucosa. In contrast, since the level of PGD₂ was measured by radioimmunoassay, it is also possible that this mediator was generated and subsequently metabolized to products undetectable by the assay used, causing us to underestimate the amount produced. Continuing our studies for longer periods of time with improved assay techniques will be necessary to pursue this question.

Other inflammatory cells and mediators may contribute to priming. Eosinophils release major basic protein, which can damage the mucosal barrier,³⁶ permitting antigen to penetrate more readily to submucosal cells. Major basic protein also can reduce ciliary activity and, thus, increase the time the nasal mucosa is exposed to pollen antigens. In the model presented,

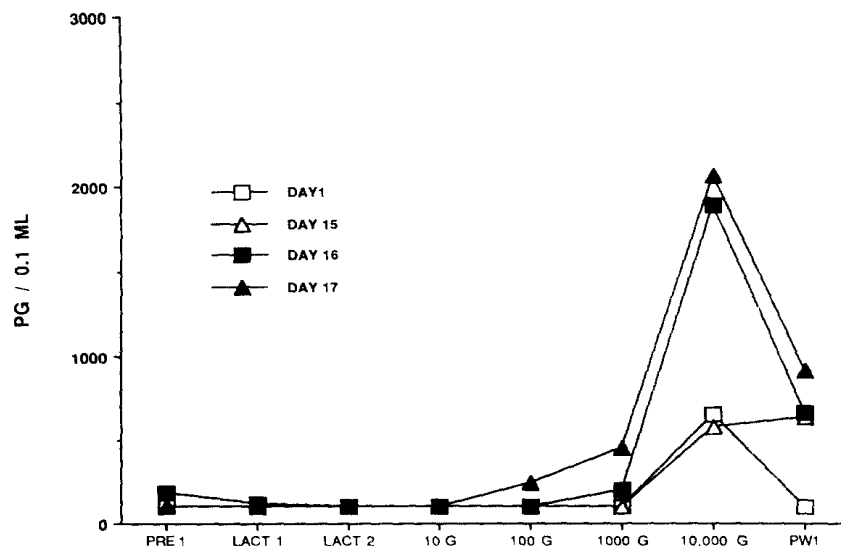


FIG. 6. Results for level of LTC₄ in recovered nasal lavages. Symbols same as in Fig. 1.

the lavages probably prevent this from being a major factor. Eosinophils also have low-affinity IgE receptors and might be stimulated directly by antigen on the priming days.³⁷ Furthermore, eosinophils contain enzymes that can influence the metabolism of mediators produced. Because of the limited amount of recovered lavage fluid, we were unable to assess the metabolism of the mediators measured. In studies during the late reaction, most of the sulfidopeptide leukotrienes were leukotrienes D₄ and E₄, in contrast to the early reaction in which LTC₄ predominates.¹⁸ Other cells, such as neutrophils and their metabolic products, could also participate in priming.

Although infiltrating cells should not be ignored, priming could be explained by other mechanisms. Mediators released during the initial reaction could affect the integrity of the mucosa, increase the releasability of resident cells, or increase the sensitivity of the end organs. For example, in the lung, hypersensitivity to histamine occurs after a late reaction and persists for days.³⁸ In the nose, similar increases in sensitivity to histamine after antigen exposure have been reported.^{27, 28} Limited evidence suggests that cyclooxygenase inhibitors can prevent this increase in reactivity, suggesting that a prostaglandin contributes to hyperreactivity.³⁹

In sum, additional information relating to the pathogenesis of priming is presented. An inflammatory cell influx occurs before priming, and the level of histamine increases during the first day of challenges in which an augmented clinical response occurred. On the second priming day, the levels of histamine,

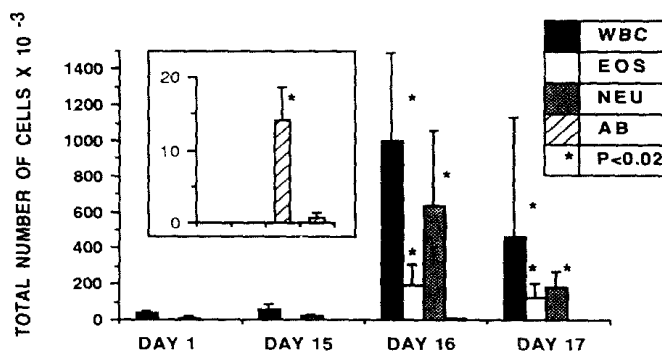


FIG. 7. The pattern of cells recovered in nasal lavages performed before the nasal challenge. WBC, white blood cells; EOS, eosinophils; NEU, neutrophils; AB, alcan blue-positive cells. Because the number of alcan blue-positive cells represents a small percentage of the total cells, an insert with a different scale was used to display the data.

TAME-esterase activity, kinins, and PGD₂ increased during the augmented clinical response. Although these factors help explain priming, additional mechanisms may be invoked. Epithelial cell activation may contribute to inflammation or the integrity of the epithelium may be compromised, allowing greater access to submucosal cells. Neuronal changes may occur, as suggested by changes in sensitivity to methacholine and histamine. Receptors for inflammatory mediators may change during the hours after the first challenge, influencing positively or negatively the response to released mediators. Also, mediators present before reexposure to antigen may act synergistically

with the ones released during reexposure or the target cells may become hyperresponsive. Inhibitor mechanisms of the allergic response may be removed. Priming could easily be a combination of some or all of these findings. Prevention of priming should be useful in the treatment of hay fever.

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Comparison between number of basophils, blood histamine, and histamine release in cancer and noncancer patients

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In cancer patients with primary tumor with or without metastasis or metastasis alone, by comparison with healthy subjects and noncancer patients, the decrease in blood histamine levels is due to a decrease in total basophil number. These basophils have a normal content of histamine (1 to 2 pg per basophil) and are able to release histamine. The percentage of anti IgE-induced histamine release is not significantly different than in noncancer patients. The scarcity of basophils in cancer patients is not due to a leukopenia. Blood histamine levels and total basophil number are normal in patients after successful excision of their primary tumor without metastasis. (J ALLERGY CLIN IMMUNOL 1989;84:501-6.)

Numerous clinical surveys have demonstrated that the atopic population has a decreased risk of malignancy and that a decreased prevalence of immediate hypersensitivity has been observed in populations with cancer.¹⁻⁶ The inverse relationship between anaphylaxis and malignant tumors is also supported by experimental data.⁷ In tumor-bearing mice, the tumor growth is accompanied by a decrease in histamine availability. In cancer patients, we have observed a decreased skin response to intradermal histamine.⁸

Finally, we have demonstrated that in patients with solid malignant tumors, with or without metastases or

with metastases alone, blood histamine levels were significantly lower than in patients without cancer.⁹

As blood histamine is quite entirely contained in basophils, it was interesting to evaluate the number of basophils to know if the decrease in blood histamine levels was due to a decrease in basophil number and/or a decrease in histamine content/basophil.

MATERIAL and METHODS

Patients

To take into consideration possible diurnal variations in blood cell count¹⁰ and in blood histamine levels, blood was always obtained between 9 and 10 AM.

Patients with cancer. All patients were hospitalized for nutritional problems and medical supervision before or after surgical treatment (tumor resection or palliative intervention). Patients who had received cancer chemotherapy or radiotherapy during the preceding 2 months were excluded from this study as well as patients receiving corticosteroids.¹¹

Clinical status allowed the division into four groups:

Group 1. Presence of unresected primary tumor without

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